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Review

The type 4 subfamily of P-type ATPases, putative aminophospholipid translocases with a role in human disease

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Abstract

The maintenance of phospholipid asymmetry in membrane bilayers is a paradigm in cell biology. However, the mechanisms and proteins involved in phospholipid translocation are still poorly understood. Members of the type 4 subfamily of P-type ATPases have been implicated in the translocation of phospholipids from the outer to the inner leaflet of membrane bilayers. In humans, several inherited disorders have been identified which are associated with loci harboring type 4 P-type ATPase genes. Up to now, one inherited disorder, Byler disease or progressive familial intrahepatic cholestasis type 1 (PFIC1), has been directly linked to mutations in a type 4 P-type ATPase gene. How the absence of an aminophospholipid translocase activity relates to this severe disease is, however, still unclear. Studies in the yeast *Saccharomyces cerevisiae* have recently identified important roles for type 4 P-type ATPases in intracellular membrane- and protein-trafficking events. These processes require an (amino)phospholipid translocase activity to initiate budding or fusion of membrane vesicles from or with other membranes. The studies in yeast have greatly contributed to our cell biological insight in membrane dynamics and intracellular-trafficking events; if this knowledge can be translated to mammalian cells and organs, it will help to elucidate the molecular mechanisms which underlie severe inherited human diseases such as Byler disease.

Keywords: Type 4 P-type ATPase; Aminophospholipid translocase; Phospholipid asymmetry; Byler disease; Membrane- and protein-trafficking; Yeast *Saccharomyces cerevisiae*

1. The P-type ATPase superfamily

The P-type ATPase superfamily is an evolutionary conserved, large family of proteins of which members are widely expressed in both prokaryotes and eukaryotes, including bacteria, yeast, parasites, nematodes, insects, plants, and animals [1–5] (See also the on-line 'P-type ATPase database' by Axelsen, K.B., http://www.patbase.kvl.dk). P-type ATPases are integral membrane proteins which, in most cases, mediate the ATP-dependent transport of small cations across biological membranes, including those of intracellular organelles [5,6]. The first P-type ATPase activity (Na^+/K^+-ATPase) was discovered in 1957 by Jens C. Skou who was awarded the Nobel Prize in Chemistry in 1997 for his work on the abundant Na^+/K^+-ATPase [7,8]. Following this finding, a whole range of different P-type ATPases have been identified with varying protein topologies and substrate specificities. In general, P-type ATPases are important in the generation of cation gradients which make these proteins indispensable in the regulation of cell volume, excitability of nerve cells, muscle contraction, intracellular pH, acid secretion in the stomach, uptake of substrates, and signaling pathways.

Based on phylogenetic analysis, this large family is divided into 5 major subfamilies, each unique in their class of substrates and in subfamily-specific sequence motifs [1–3]. In general, the type 1 ATPases transport heavy metals such as copper, cadmium, and zinc. The type 2 subfamily transports non-heavy metal cations and includes proteins like the Na^+/K^+-, H^+/K^+-, and Ca^{2+}-ATPases. The type 3

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family includes ATPases only expressed in plants and fungi which are involved in the transport of magnesium- and proton-ions. Type 4 subfamily members are exclusively expressed in eukaryotic cells and transport phospholipids (see below) [9–12]. Finally, of the recently identified type 5 subfamily (exclusively expressed in eukaryotic cells), only one member has been identified yet which is implicated in cellular calcium homeostasis and endoplasmic reticulum function in yeast [13–15]. All P-type ATPases share a common topology; usually 10 transmembrane domains, NH_2- and COOH-termini protruding into the cytoplasm, and a large intracellular loop harboring P-type ATPase-specific sequences and an ATP-binding site. The core of all P-type ATPases contains a conserved stretch of seven amino acids with the P-type signature sequence 'DKTGT[L,I,V,M][T,I,S]'. This motif includes an aspartic acid residue ('D') which is essential in the reaction cycle of the protein. The mechanism via which substrates are pumped is based on the E1E2 model, in which ATP-mediated phosphorylation, substrate binding, and subsequent dephosphorylation of the invariant aspartic acid are coupled to a conformational change of the transporter [6,16]. The phosphotransfer reaction drives the transport cycle of these proteins, hence the name P-type ATPase. Although phosphotransfer remains the driving force for these transporters, the E1E2 model is outdated, and recently, a revised model has been proposed [17,18]. Briefly, in the E1E2 model, only two conformational states of the protein are recognized; the revised model, however, recognizes at least four conformational changes, all of which are supported by a large body of biochemical and structural data (reviewed in [17,18]).

Type 4 P-type ATPases are clearly deviant from the other subfamilies in that they are thought to translocate phospholipids (rather than cations) from the outer- and inner leaflet of membrane bilayers [9,11,19–23]. Studies in yeast have recently demonstrated an association between type 4 ATPase-mediated phospholipid translocase activity and vesicular (protein) trafficking [11,20,24,25]. Furthermore, members of this subfamily have been implicated in human disease. The molecular mechanisms underlying these disease phenotypes are, however, still unclear. The scope of this review is to highlight the role of type 4 P-type ATPases in human disease and to relate this to recent findings in yeast in which an important function for type 4 ATPases in intracellular trafficking has been demonstrated.

2. Aminophospholipid translocases and membrane asymmetry

Biological membranes are bilayers which are composed of a broad range of glycerolipid and sphingolipid species. These lipid species are asymmetrically distributed between the inner and the outer leaflet of the bilayer [26–28]. Phospholipids tend to equilibrate between the two leaflets of bilayers, albeit at a slow rate (the order of magnitude of the movement across the bilayer tends to be hours in artificial membranes (reviewed in [29])). In living cells, this process is accelerated by fusion and pinching of vesicles to and from membranes. The asymmetry therefore needs to be actively maintained. Three classes of proteins are involved in regulating phospholipid distribution in membrane bilayers, i.e., floppases, flippases, and scramblases [19,20,25,30–35]. Phospholipid asymmetry is maintained by floppases and flippases, ATP-dependent proteins that translocate specific phospholipid species from the inner to the outer leaflet of the bilayer and vice versa, respectively. Scramblases are bidirectional activities with little specificity that, upon activation, dissipate phospholipid asymmetry. Scramblase activities have been identified in plasma membranes and in membranes of the endoplasmic reticulum and the Golgi apparatus [22,34,36–39]. In many cells, these activities are dependent on elevation of cytosolic calcium concentrations, e.g., in cells involved in blood coagulation [34]. However, in apoptotic cells, it seems that another mechanism (rather than calcium) is involved in activation of the scramblase [40].

In most eukaryotic cells, phosphatidylcholine (PC) and sphingolipids (i.e., glycolipids and sphingomyelin (SM)) are abundant in the outer leaflet of the plasma membrane and in the luminal leaflet of endo- and exocytic vesicles. Proteins of the ATP-binding cassette (ABC) transporter superfamily have been implicated in the outward transport, i.e., 'flopping' of choline-containing phospholipids [41]. Mice in which the gene encoding the liver-specific Mdr2 protein (Abcb4) has been knocked out do not secrete PC into the canalicular lumen [42]. The human ortholog, MDR3 (ABCB4) protein, likewise specifically translocates PC (as well as its fluorescent analogs) from the inner to the outer leaflet of the bilayer when expressed in mouse fibroblasts or pig epithelial (LLC-PK1) cells [42–44]. MDR1 (ABCB1) protein, a broad-range xenobiotic transporter, also translocates choline-containing lipids, including short-chain analogs of PC, glucosylceramide, and platelet-activating factor [44,45]. In addition, MRP1 (ABCC1) protein has been implicated in the outward transport of fluorescent analogs of phosphatidylserine (PS) and SM [46,47].

The aminophospholipids PS and phosphatidylethanolamine (PE) localize to the cytosolic leaflet of bilayers and their distribution is maintained by flippases or aminophospholipid translocases (APLT) in a process termed 'flipping'. Obviously, maintenance of phospholipid asymmetry must be a physiologically important process as the cell invests energy to specifically distribute these distinct phospholipid species. Indeed, disturbance of membrane phospholipid asymmetry, i.e., exposure of PS and/or PE to the outer leaflet of the bilayer (scrambling) is associated with many physiological and pathological events. Phos-

pholipid scrambling interferes with lipid–protein and protein–protein interactions which affect protein function involved in signal transduction pathways, cellular morphology, and activity of membrane receptors and transport proteins (reviewed in [32,48]). Furthermore, PS and/or PE exposure on the outer leaflet is essential for platelet activation, triggers the apoptotic pathway, and is required for membrane fusion events, including endo-, exo-, and phagocytosis [22,48–52]. Thus, maintenance of phospholipid asymmetry by aminophospholipid translocases is a fundamental process which is of vital importance for normal cell function.

The first aminophospholipid translocase activity was described in 1984 in erythrocyte membranes by Seigneuret and Deveaux [53]. In 1989, Zachowski et al. [12] identified an ATP-dependent translocase activity in chromaffin granules from bovine adrenal glands; spin-labeled phospholipid analogs of PS were specifically translocated from the luminal to the cytoplasmic leaflet of these organelle membranes. The authors hypothesized that an aminophospholipid translocase activity in these Golgi-derived vesicles was necessary to generate fusion-competent, exocytic, vesicles. A few years later, Auland et al. [9] purified and reconstituted a 110-kDa, Mg^{2+}-dependent, ATPase from human erythrocyte membranes; they demonstrated a 110-kDa-mediated transport activity similar to the activity in chromaffin granules, i.e., translocation specific for spin-labeled PS and, to a lesser extent, PE.

In 1996, the gene encoding the chromaffin granular aminophospholipid translocase activity, ATPase II (currently known as ATP8A1), was cloned from a bovine adrenal medulla and thymus cDNA library [11]. In the same study, Drs2p, the closest yeast *Saccharomyces cerevisiae* homolog of this protein, was characterized as a plasma membrane aminophospholipid translocase activity; using *Drs2* mutant yeast cells, it was demonstrated that Drs2p was involved in the inward translocation of a fluorescently (NBD)-labeled PS analog (NBD-PS) across the plasma membrane. Although the Drs2p-mediated plasma membrane NBD-PS translocation activity is controversial (see below and [54–56]), both ATPase II and Drs2p were the first members of a novel subfamily of the P-type ATPase superfamily, the type 4 subfamily [1,2,19,21,22,57]. Thus far, aminophospholipid translocase activities have been demonstrated in many mammalian cell types and subcellular membranes [19]. Since ATPase II is a chromaffin granular protein and is not expressed in every tissue, it is tempting to speculate that other P-type ATPases of the type 4 subfamily account for similar activities in other tissues and/or subcellular locations, including the plasma membrane [21,58].

3. The mammalian type 4 subfamily of P-type ATPases

The type 4 subfamily constitutes a relative new branch on the phylogenetic tree of P-type ATPases and, as discussed above, members are putative aminophospholipid translocases. The idea that mammalian members of this subfamily have a role in the translocation of (amino)-phospholipids is mainly based on the characterization of their yeast homologs (see below). Database searches and phylogenetic analyses have identified 14 mammalian type 4 P-type ATPases [1,21,22] (see Table 1 and Fig. 1). Type 4 ATPases share several specific amino acid stretches and differ from most other P-type ATPases in their substrate binding domain, which is usually formed by one or more of the ten transmembrane helices [1,4,6]. In transmembrane domains 4 and 6, several metal ion-interacting amino acids (present in most cation-transporting P-type ATPases from other subfamilies) are replaced by hydrophobic amino acids which may interact with hydrophobic moieties of amphipathic substrates, including phospholipids [1,4,6,11]. The type 4 subfamily is divided into 4 classes, 1, 2, 5, and 6 of which members show high sequence similarities, ranging from 23 to 37% sequence identity between proteins of the 4 classes and 43–74% sequence identity between proteins within classes. Class 1 members are subdivided into subclasses 1-Σ (ATP8A1 (ATPase II) and ATP8A2) and 1-Φ (ATP8B1 and its close homologs ATP8B2, ATP8B3, and ATP8B4) [21,22,59]; ATP9A and ATP9B are class 2 proteins; class 5 proteins are ATP10B, -C, and -D; ATP11A, -B, and -C are class 6 proteins (see Table 1 and Fig. 1). The distinction into classes is based on sequence identity rather than on biochemical activities or substrate specificities. Indeed, the biochemical activity and substrate specificity of most of these mammalian proteins remain to be elucidated. Ding et al. [60] were the first to demonstrate a convincing correlation between biochemical activity and substrate specificity of mammalian ATPase II (ATP8A1). After purification of 4 bovine ATPase II isoforms and subsequent reconstitution into a defined lipid environment, the authors demonstrated an aminophospholipid (PS and PE)-specific activation of the ATPase activity of these proteins; these data strongly suggest that ATP8A1 indeed is an aminophospholipid translocase [12,60]. Many of the type 4 ATPase genes encode different splice variants and are ubiquitously expressed, with almost every protein in the brain (see Table 1). The only exception is *ATP8B3* which is specifically expressed in the testis [59]. Recently, Wang et al. [61] have shown that the mouse ortholog of ATP8B3 is exclusively expressed in the acrosomal region of the head of spermatozoa; Atp8b3 has been implicated in the capacitation process, an event in which sperm cells are being prepared for binding to and penetration of the zona pellucida. Since binding to the zona pellucida requires a scrambling-induced destabilization of the sperm plasma membrane, the authors have suggested a role for Atp8b3 in the sperm-specific translocation of aminophospholipids [61]. All 14 human ATPases have orthologs in the mouse, which gives an excellent opportunity to study the physiological relevance and putative disease-

Table 1
Tissue distribution and chromosomal localization of human type 4 P-type ATPases; when human distribution is not known, mouse distribution is depicted in italic

Class	Gene	Protein name	Chromosomal localization	Database accession (*mouse*)	Tissue distribution (*mouse*)	Reference
1-Σ	ATP8A1	ATPase IA/ATPaseII (1164 AA)	4p14–p12	Q9Y2Q0 *(P70704)*	Ubiquitous; high in skeletal muscle, brain; not in liver, lung, testis	[58]
1-Σ	ATP8A2	ATPase IB/ML-1 (1148 AA)	13q12–13	Q9NTI2 *(P98200)*	*High in testis; low in heart, brain; not in liver, lung, kidney*	[21]
1-Φ	ATP8B1	ATPase IC/FIC1 (1251 AA)	18q21–18q22	O43520 *(NP_001001488)*	Ubiquitous; high in small intestine, pancreas; low in brain	[87]
1-Φ	ATP8B2	ATPase ID (1209)	1q21.3	P98198 *(XP_283873)*	Ubiquitous; high in brain, bladder, uterus; not in kidney, skeletal muscle	[59]
1-Φ	ATP8B3	ATPase IK (1310 AA)	19p13.3	O60423 *(NP_080370)*	Testis	[59]
1-Φ	ATP8B4	ATPase IM (1192 AA)	15q21.2	Q8TF62 *(XP_141343)*	Ubiquitous at moderate levels including, brain, liver, kidney, testis	[142]
2	ATP9A	ATPase IIA (1047 AA)	20q13.1–q13.2	O75110 *(O70228)*	Ubiquitous at moderate levels; not in spleen	[143]
2	ATP9B	ATPase IIB/Hussy-20 (1095 AA)	18q23	O43861 *(P98195)*	*Ubiquitous; high in testis; not in spleen, muscle*	[21,57]
5	ATP10A	ATPase VA (1499 AA)	15q11–q13	O60312 *(O54827)*	Ubiquitous; high in brain, kidney, lung, pancreas; not in small intestine	[78]
5	ATP10B	ATPase VB (1461 AA)	5q34	O94823 *(CAI26159)*	Low in brain and testis	[144]
5	ATP10D	ATPase VD (1426 AA)	4p12	Q9P241 *(NP_700438)*	Ubiquitous, moderate in liver, kidney, spleen, ovary; low in brain	[145]
6	ATP11A	ATPase IH (1134 AA)	13q34	P98196 *(P98197)*	Ubiquitous; moderate in liver, heart, kidney, muscle; low in brain, spleen	[146]
6	ATP11B	ATPase IF (1177 AA)	3q27	Q9Y2G3 *(XP_358349)*	Ubiquitous (low); moderate in kidney	[147]
6	ATP11C	ATPase IG (1132 AA)	Xq27.1	NP_775965	Ubiquitous; high in liver, pancreas, kidney; low in brain, skeletal muscle	[86]

Gene and protein names are indicated (amino acid number of each protein is depicted in between brackets). Mouse accession numbers are enclosed in pharentheses and in italic. For mouse tissue distribution and chromosomal localization, see also references [21,22,57].

causing mutations of these proteins in vivo (see Table 1) [21,22].

4. P-type ATPases and human disease

Up to now, several human inherited diseases have been described which are caused by mutations in genes encoding P-type ATPases. The most extensively studied disorders are caused by mutations in genes encoding ATPases from the type 1 and 2 subfamilies, including copper-, calcium-, and Na^+/K^+-ATPases. For the type 4 subfamily, encoding the putative phospholipid translocases, a few inherited disorders have been mapped to loci harboring type 4 ATPase genes. Up to now, only one inherited disorder, Byler disease or progressive familial intrahepatic cholestasis type 1 (PFIC1) has been directly linked to mutations in a type 4 P-type ATPase gene. How absence of a putative aminophospholipid translocase activity relates to this severe disease is, however, still unclear. The etiology of PFIC1 disease and the relation of the phenotype with a putative aminophospholipid translocase activity will be addressed below. Inherited disorders associated with the type 5 subfamily remain to be identified. Thus, defective P-type ATPase activities underlie severe genetic disorders which underscores their importance in basic cellular processes and maintenance of cellular homeostasis.

4.1. Type 1 and 2 subfamily inherited disorders

The best characterized diseases caused by defective type 1 P-type ATPases include Menkes disease, an X-linked recessive disorder, and Wilson disease, an autosomal recessive disorder (reviewed in [62]). These disorders are caused by mutations in the *ATP7A* and *ATP7B* gene, respectively, and result from systemic copper deficiency (due to a defect in intestinal copper absorption) and copper accumulation (predominantly in the liver due to impaired hepatobiliary excretion), respectively [63–68]. Brody disease, an autosomal recessive myopathy, is caused by mutations in the *ATP2A1* gene encoding the fast-twitch skeletal muscle sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA1), and results in impaired muscle relaxation [69]. Darier–White disease is an autosomal dominant skin disease caused by mutations in *ATP2A2* which encodes a Ca^{2+}-ATPase highly expressed in the endoplasmic reticulum of keratinocytes (SERCA2) [70]. Both SERCA1 and 2 mediate the transport of Ca^{2+} from the cytosol to the lumen of the sarco-/endoplasmic reticulum which is important in the regulation of contraction-relaxation and of epidermal growth and differentiation, respectively [69–71]. Hailey–Hailey disease is an autosomal dominant defect in keratinocyte adhesion caused by mutations in another endoplasmic reticulum Ca^{2+}-ATPase gene, *ATP2C1* [72]. Mutations in the gamma-subunit of the Na^+/K^+-ATPase, *FXYD2*, results in a renal hypomagnesia due to

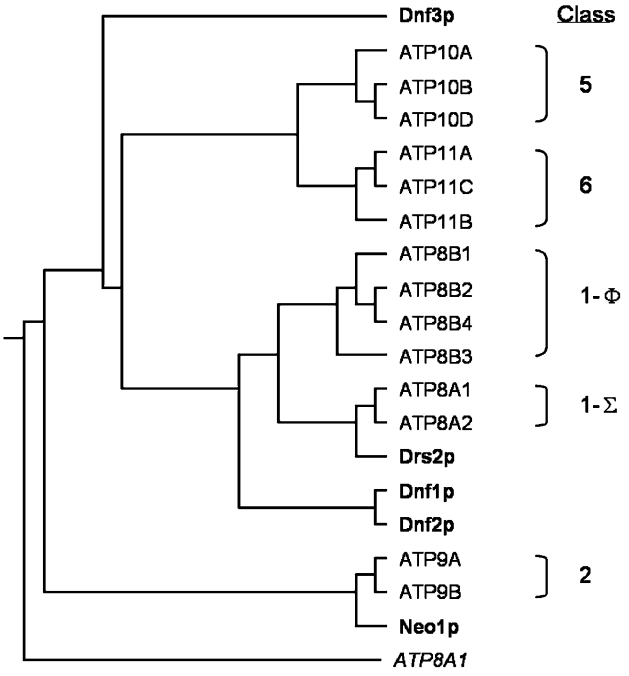

Fig. 1. Phylogenetic analysis of the human and yeast *Saccharomyces cerevisiae* (in bold) type 4 ATPases. The figure shows the relationship between the full-length proteins of this family (in comparison to ATP8A1 (in italic)) in a dendrogram. The dendrogram was generated using ClustalW analyses. For clarity, the protein names as depicted in Table 1 have been omitted from the dendrogram and are replaced by the nomenclature used for the genes. Classes for the human type 4 ATPases are depicted. Protein accession numbers for the human proteins are from Table 1. Database accession numbers for the other proteins: ATP8A1, Na+/K+-ATPase alpha-1 chain (P05023), Dnf1p (P32660), Dnf2p (Q12675), Dnf3p (Q12674), Drs2p (P39524), Neo1p (NP_012216).

misrouting of the protein, and as a consequence, a renal magnesium loss [73]. The molecular mechanisms underlying above-mentioned P-type ATPase-associated diseases have been largely elucidated.

4.2. Type 4 subfamily inherited disorders

Thus far, only a few diseases have been described which are associated with loci harboring type 4 P-type ATPase genes. The *ATP8A2* gene locus was reported to be frequently deleted in tumorigenic malignancies, however, thus far, no relation between defective phospholipid translocase activity and tumorigenesis has been demonstrated [74]. Angelman or 'happy puppet' syndrome is a neurological disorder characterized by frequent smiling, bursts of laughter, mental retardation, hyperactivity, and epilepsy, and is a disorder in which genetic imprinting plays a role (reviewed in [75,76]); this syndrome has been associated with mutations or deletions in a maternally-derived, chromosomal region which includes the *ATP10A* (formerly known as *ATP10C*) and *UBE3A* genes [77,78]. *UBE3A* encodes the ubiquitin-protein ligase E3A, an enzyme involved in the ubiquitination of proteins. It is highly likely, however, that the syndrome relates to the *UBE3A* gene rather than to the *ATP10A* gene; transgenic mice in which the maternal *Ube3aA* gene was knocked out, phenotypically resembled the human patients [79]. Mice heterozygous for maternally-inherited deletions of *Atp10a* (or *pfatp* (p-locus fat-associated ATPase)), on the other hand, displayed increased body fat content when compared to heterozygous mice which inherited the paternal *Atp10a* [80–82]; thus, the mouse ortholog of *ATP10A, Atp10a*, is implicated in lipid metabolism, obesity, type 2 diabetes, and non-alcoholic fatty liver disease rather than in neurological aberrations. Similarly, *Atp10d*, which belongs to the same class as *Atp10a* (see Table 1 and Fig. 1), has also been implicated in lipid metabolism [83]; of 15 inbred mouse strains, only the C57BL/6J strain contained a premature stop codon in the *Atp10d* gene, resulting in a non-functional protein. Interestingly, C57BL/6J mice are predisposed to develop obesity, hyperglycemia, hyperinsulinemia, and hypertension when fed a high-fat diet (this unlike controls i.e., A/J mice) [84,85]. Although for both Atp10d and Atp10a, the data are circumstantial, it is tempting to speculate that these P-type ATPases fulfill important activities in the regulation of lipid metabolism. Recently, *ATP11C* has been mapped to a chromosomal region, Xq27, associated with X-linked inherited disorders, including hypoparathyroidism, albinism-deafness, and thoracoabdominal syndrome [86]. If and how this ATPase contributes to these disease phenotypes remains to be demonstrated.

Thus far, only one human disease has been directly correlated with mutations in a gene encoding a type 4 P-type ATPase. Mutations in the *ATP8B1* (or *FIC1*) gene are the cause of Byler disease or progressive familial intrahepatic cholestasis type 1 (PFIC1), a disease which will be discussed below [87].

5. Byler disease or progressive familial intrahepatic cholestasis type I (PFIC1)

Byler disease or PFIC1 is a rare autosomal recessive disorder, first described in the Amish kindred in 1969 by Clayton et al. [88]. The disease primarily manifests as a chronic intrahepatic cholestasis which progresses to severe, end-stage liver disease before adolescence [88–92]. A less severe clinical phenotype associated with mutations in *ATP8B1* is benign recurrent intrahepatic cholestasis (BRIC) [93]. In BRIC disease, the cholestatic periods are recurrent and resolve spontaneously, without leaving any detectable liver damage. Recently, an inventory of mutations in the *ATP8B1* gene has been described which relates the type and location of the mutation to the clinical manifestation of PFIC1/BRIC disease [94]. Biochemically, PFIC1/BRIC patients are characterized by impaired hepatobiliary bile salt secretion, elevated serum bile salts, bilirubin (jaundice), liver damage enzymes including alkaline phosphatase and normal serum cholesterol. While in most cholestatic disorders serum gamma-glutamyltranspeptidase activity is

high, this activity is only marginally increased in PFIC1/BRIC patients. Liver morphology is characterized by bile plugs, central vein sclerosis and portal tract expansion, inflammation, and fibrosis [91,95–98]. Furthermore, giant cell transformation and hepatocyte ballooning are prominent. Ultrastructural abnormalities include loss of microvilli, dilated canaliculi, and proliferation of pericanalicular microfilaments. The most pronounced ultrastructural abnormality is the structure of the bile of PFIC1/BRIC patients, termed 'Byler bile' which has a coarsely granular appearance (in contrast to normal bile which has an amorphous structure) [91,95–98]. Apart from a hepatic phenotype, also extrahepatic abnormalities have been described, including watery diarrhea, pancreatitis, and in some cases, hearing loss and elevated sweat chloride concentrations [91,99]. Many PFIC1 patients need to undergo orthotopic liver transplantation before adolescence. Although liver transplantation relieves the cholestasis and jaundice, the diarrhea and elevated sweat chloride concentrations remain; furthermore, after liver transplantation, patients develop hepatic steatosis [91,100]. These post-transplantation phenotypes implicate an important contribution of intestinal ATP8B1 protein in the etiology of PFIC1 disease. Indeed, ATP8B1 protein is expressed in many tissues including liver, pancreas, small intestine, urinary bladder, stomach, and prostate [87]. In these tissues, ATP8B1 localizes to the apical membrane of epithelial cells, including hepatocytes, cholangiocytes, enterocytes, gastric pit cells, and acinar cells of the pancreas [101–103].

At this moment, it is still not clear how absence of the ATP8B1 protein relates to the cholestatic phenotype observed in PFIC1/BRIC disease. Obviously, absence of ATP8B1 has a major impact on bile salt homeostasis, and predominantly on the hepatobiliary excretion of bile salts. Apart from increased systemic bile salt levels and impaired hepatobiliary bile salt excretion, analyses of PFIC1 bile have indicated that the ratio of the primary bile salts has changed: substantially reduced levels of chenodeoxycholic acid and increased concentrations of cholic acid have been reported [91,96,98,104,105]. This led to the hypothesis that ATP8B1 is involved in the hepatobiliary transport of bile salts, specifically chenodeoxycholic acid [104]. However, interruption of the enterohepatic circulation of bile salts in PFIC1 patients resulted in a remarkable normalization of serum bile salts with restored hepatobiliary output of chenodeoxycholic acid [98,106–108]. This indicates that the bile salt transport defect in PFIC1 disease is not a direct consequence of impaired ATP8B1 function. Rather, absence of ATP8B1 may indirectly affect the activity of the major bile salt transporter in the canalicular membrane of the hepatocyte, the bile salt export pump (BSEP/ABCB11).

Farnesoid X receptor (FXR) is a nuclear receptor for bile salts, and the key transcriptional regulator for genes involved in the synthesis, conjugation, and transport of bile salts, both in liver and intestine [109,110] (reviewed in [111]). FXR regulates the expression of BSEP and the intestinal apical sodium-dependent bile salt transporter, ASBT/SLC10A2, a protein involved in the intestinal absorption of bile salts [112–115]; in response to elevated bile salt levels, FXR is activated which subsequently induces BSEP expression in the liver and represses ASBT expression in the intestine. Recently, two groups reported on a down-regulation of *FXR* mRNA levels and subsequent down- and up-regulation of *BSEP* and *ASBT* mRNA, respectively, in PFIC1 patients [116,117]. Both groups suggested that impaired ATP8B1 function resulted in reduced FXR expression and subsequent impairment of hepatobiliary and intestinal bile salt transport, which is causative for the cholestatic phenotype. However, studies in patients with impaired BSEP function, which like PFIC1 patients have elevated serum bile salt levels, also have reduced *FXR* expression levels implicating that the drop in *FXR* levels is secondary to the cholestasis [118]. In addition, studies in the mouse model for Byler disease, the Atp8b1$^{G308V/G308V}$ mutant mouse, which also has elevated serum bile salt levels, did not reveal a significant change in *Fxr* expression levels unless serum bile salt levels were highly increased by bile salt feeding [119].

As a member of a subfamily of putative P-type aminophospholipid translocases, ATP8B1 may have a crucial role in maintaining membrane integrity. Impaired ATP8B1 protein function in PFIC1 disease may affect the composition of the canalicular membrane, which subsequently affects the activity of proteins involved in bile formation, including BSEP protein. It is noteworthy that the polarized human hepatoma cell line HepG2 expresses an aminophospholipid translocase activity in the apical membrane domain [120]; indeed, ATP8B1 is expressed in HepG2 cells, albeit at a low level (unpublished data, CCP), however, it remains to be established if this translocase activity is ATP8B1-derived. Previously, Ujhazy et al. [102] have shown a relation between ATP8B1 expression and uptake of a fluorescent aminophospholipid analog, NBD-labeled phosphatidylserine (NBD-PS). ATP8B1 was expressed in a mutant CHO-K1 cell line (termed UPS-1) which was impaired in the non-endocytic uptake of NBD-PS analogs [121]. When these cells were incubated with NBD-PS for 10 min at 37 °C, an ATP8B1-dependent increase in intracellular NBD-PS was observed [102]. This may be due to translocation of the NBD-PS from the outer to the inner leaflet of the plasma membrane, which indicates that ATP8B1 indeed has an aminophospholipid translocase activity. However, a more direct demonstration of such an activity would be favorable, as these data do not rule out an indirect effect of ATP8B1 overexpression on NBD-PS uptake. For instance, ATP8B1 could activate an endogenous translocase activity, or may directly or indirectly be involved in endocytosis.

Although some type 4 ATPases are associated with lipid metabolism, it remains unclear what molecular mechanisms underlie the above-mentioned inherited diseases. However, recent studies in yeast have shed light on how P-type

ATPase-mediated APLT activity relates to cellular processes, including protein trafficking and endocytosis [11,20,24,25]. This will be discussed below.

6. What can we learn from yeast APLT activities?

The yeast *S. cerevisiae* expresses 5 members of the type 4 subfamily of ATPases, including Drs2p, Dnf1p, Dnf2p, Dnf3p, and Neo1p (see Fig. 1) [1,4,20,122]. All proteins have been implicated, either directly or indirectly, in the translocation of phospholipids and participate in intracellular vesicular-trafficking pathways. Studies in yeast strains defective in single or multiple type 4 ATPases have accumulated a large body of data which implicate these proteins in the biogenesis of transport vesicles and thus the establishment of cellular polarity. Furthermore, these proteins act in distinct or overlapping pathways (see Fig. 2).

Drs2p shares highest amino acid identity with human class 1-Σ members (44%), including ATP8A1, and was initially implicated in aminophospholipid translocation in the plasma membrane of yeast cells [11]. Later studies, however, demonstrated that the protein and translocase activity predominantly localized to the *trans*-Golgi network (TGN) rather than to the plasma membrane [10,54–56,122,123]. Using a Drs2p mutant strain (*Δdrs2*) expressing a temperature-sensitive Drs2p (*Δdrs2*^{ts}) or a wild-type Drs2p (*Δdrs2*^{WT}), Natarajan et al. [55] have elegantly shown that Drs2p specifically translocates fluorescently (NBD)-labeled PS from the luminal to the cytosolic leaflet of TGN membranes; translocation was head-group specific as no translocation of NBD-PC or -PE was observed. It should be emphasized that the *Δdrs2*^{ts} and *Δdrs2*^{WT} strains were also mutated for all 3 Dnf proteins in order to eliminate redundancy in overlapping pathways (see below; Fig. 2). Drs2p is important in the formation of exocytic, clathrin-coated transport vesicles at the TGN; *Δdrs2* cells and *Δdrs2* cells expressing ATPase-deficient Drs2p have reduced amounts of vesicles compared to cells expressing the wild-type protein [122–124]. Furthermore, Drs2p is involved in the TGN-to-vacuole transport of alkaline phosphatase. Using yeast mutants defective in PS synthesis (*Δcho1*), it was demonstrated that translocation of PS is not specifically required for proper Drs2p function: *Δcho1* cells did not resemble the phenotype of *Δdrs2* cells with regard to the formation of exocytic transport vesicles. Recently, it has been suggested that Drs2p cycles between the TGN, late endosomes, and the plasma membrane and is involved in the trafficking of Dnf1p, a plasma membrane APLT (see below and [125]); in *Δdrs2* cells, the localization of Dnf1p was in cytoplasmic punctate structures rather than in the plasma membrane. This observation may explain the initial finding by Tang et al. [11] who reported an impaired plasma membrane APLT activity in *Δdrs2* cells while Drs2p is primarily localized within the cell. The absence of plasma membrane APLT activity would then be caused by impaired Drs2p-mediated targeting of Dnf1p. *Δdrs2* cells accumulate membranous, Golgi-derived structures called Berkeley bodies, which show remarkable structural similarity to autophagosomes [122,123,126,127]. The phenotype of

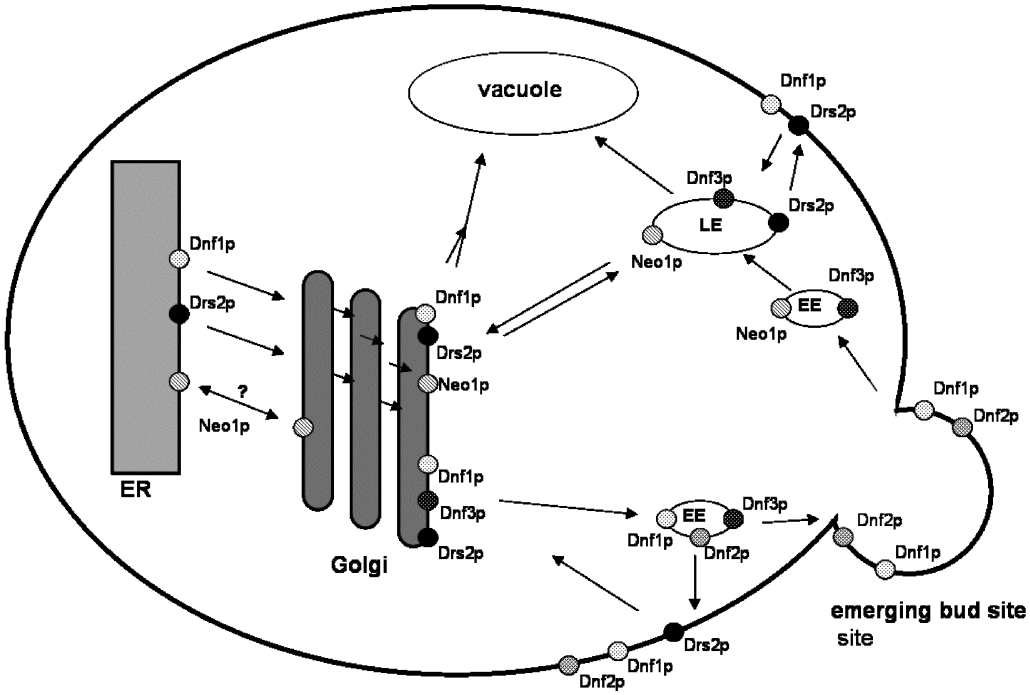

Fig. 2. Schematic and simplified representation of a yeast cell depicting the localization of type 4 ATPases and the vesicular-trafficking pathways in which these proteins participate (for details, see text). For clarity, interacting proteins have been omitted. EE, early endosome; LE, late endosome. Localization of the proteins and the pathways involved are summarized from literature [10,122,123,125,129,130,135,138]. Figure adapted from reference [20].

Δdrs2 cells became more severe when Dnf1p was mutated as well (*Δdrs2Δdnf1)*; while in *Δdrs2* cells, alkaline phosphatase predominantly localized to the vacuolar membrane, *Δdrs2Δdnf1* cells displayed a strongly reduced vacuolar staining for alkaline phosphatase, suggesting a major defect in the TGN-to-vacuole pathway [122]. Also, cell growth was severely impaired and the amount of Berkeley bodies was dramatically increased in *Δdrs2Δdnf1* cells, suggesting a more severe defect in protein trafficking in these cells. These phenotypes were not observed in *Δdnf1* single mutant, nor in *Δdnf1Δdnf2Δdnf3* triple mutant cells, suggesting that Drs2p and Dnf1p act in overlapping pathways in which Dnf1p plays a minor, but additive role [122].

Dnf1p and Dnf2p share highest amino acid sequence identity with human class 1-Σ (e.g., ATP8A1) and class 1-Φ (e.g., ATP8B1) type 4 ATPases (36–38%). Both proteins predominantly localize to the plasma membrane, appearing as punctate structures with a concentration at sites of polarized growth (i.e., buds, emerging bud sites and the mother–daughter neck of dividing cells [10,122,125] (see Fig. 2). In addition, some punctate cytoplasmic staining has been described for both Dnf1p and Dnf2p, possibly representing endocytic and/or exocytic structures, an observation which indicates that both proteins cycle between the plasma membrane and endomembrane systems [10,122, 125]. Pomorski et al. [10] have shown that *Δdnf1Δdnf2* double mutant cells display a similar growth phenotype as *Δdrs2* cells, i.e., reduced growth at temperatures below 20 °C, again emphasizing redundancy for these yeast type 4 ATPases. In addition, *Δdnf1Δdnf2* cells are impaired in the ATP-dependent, non-endocytic uptake of NBD-labeled glycerolipid analogs, including NBD-PE, NBD-PC, and NBD-PS; translocation of NBD-labeled sphingoid-based lipids, including sphingomyelin (NBD-SM), was not impaired in these double mutants, which indicates that the translocation defect is specific for glycerophospholipids [10]. Importantly, the authors have demonstrated that, besides a defect in NBD-labeled PE uptake, *Δdnf1Δdnf2* cells display a 2-fold increase in endogenous PE in the outer leaflet of the plasma membrane; PE exposure was even more pronounced in *Δdnf1Δdnf2Δdrs2* triple mutant cells, in which also low levels of endogenous PS were detected in the outer leaflet of the plasma membrane. Finally, the authors have shown that *Δdnf1Δdnf2Δdrs2* cells have a defect in the initiation step of both fluid-phase and receptor-mediated endocytosis. These observations strongly suggest that Dnf1p, Dnf2p, and Drs2p participate in overlapping pathways and have a role in the translocation of endogenous glycerophospholipids. Furthermore, these data link APLT activities with the endocytic pathway.

Dnf3p shares 30–36% amino acid identity with all human type 4 ATPases, and has overlapping localization profiles with Drs2p, i.e., in the TGN and endosomal compartments [10,122]. Using a green fluorescent protein-tagged v-snare protein (GFP-Snc1p), Hua et al. [122] have shown that *Δdnf1Δdnf2Δdnf3* triple mutant cells are impaired in the recycling of proteins in the endosome-to-TGN-to-plasma membrane loop. Besides Dnf3p, both Dnf1p and Dnf2p may also cycle in this pathway as these proteins have been localized to the plasma membrane and in intracellular vesicles. Milder protein-trafficking defects were observed in *Δdnf1Δdnf2*, *Δdnf1Δdnf3*, and in *Δdrs2* cells, implicating these proteins in distinct trafficking pathways. Because *Δdrs2* cells have a defect in the biogenesis of exocytic transport vesicles, it is not clear whether these cells also have a problem with the retrograde transport from endosome-to-TGN [122]. At this moment, it is not clear what the relative contribution is of all these proteins in the different trafficking pathways.

Finally, Neo1p, which shares highest amino acid identity with human class 2 type 4 ATPases (49%), is the only yeast type 4 ATPase essential for cell growth [128]. Neo1p has been localized to early and late endosomal compartments, and to a lesser extent to the TGN [10,129]. Studies in *Δneo1* cells expressing temperature-sensitive- or N-terminally-tagged (functional) Neo1p have indicated a role for Neo1p in fluid-phase- and receptor-mediated endocytosis, vacuole biogenesis, and in vacuolar protein sorting [129]. In another report, Neo1p has been implicated in retrograde vesicular transport between *cis*-Golgi and endoplasmic reticulum, however, the C-terminally-tagged Neo1p used in this study turned out to be non-functional [130]. Analogous to *Δdrs2* and *Δdrs2Δdnf1* cells, *Δneo1* cells also accumulated abnormal Golgi- and/or endosomal-derived membranous structures in the cytoplasm which morphologically resemble Berkeley bodies [129,130]. As for the other type 4 ATPases, these data suggest a role for Neo1p in vesicular protein trafficking, albeit within the endosomal/TGN system [129].

7. Molecular interactions of yeast type 4 ATPases

7.1. ADP-ribosylation factors and guanine nucleotide exchange factors

Additional evidence for a role in membrane-trafficking events comes from studies which describe molecular interactions of type 4 members with proteins important in vesicular trafficking, including ADP-ribosylation factor (ARF). ARFs are small GTPases which are regulated by guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs) (reviewed in [131]). ARFs are important in the recruitment of COPI and clathrin coat proteins which is an essential process in the initiation of vesicle formation (reviewed in [132]). COPI-coated vesicles are formed in the Golgi after recruitment of ARF-GDP and subsequent membrane binding of ARF-GTP. COPI vesicles traffic from the Golgi to the endoplasmic reticulum (ER) (and between Golgi cisternae), whereas clathrin-coated vesicles traffic from the TGN to the endosome and plasma

membrane, and from the plasma membrane to early endosomes. In yeast, Arf1p is important in the regulation of Golgi and endosome structure and function, and in the secretory pathway [133,134]. Chen et al. [123] have demonstrated that yeast Arf1p and Drs2p act in a parallel or overlapping, clathrin-involved pathway: where both *Δarf1* and *Δdrs2* cells have normal growth, *Δarf1Δdrs2* double mutants are lethal. Membrane recruitment of ARF-GDP is mediated by cytoplasmic ARFGEFs. After membrane binding of ARF-GTP, COPI coatomers bind and cargo protein assembly takes place. Recently, it has been demonstrated in yeast that the Arf1GEF Gea2p physically interacts with Drs2p, and that Gea2p localization is slightly affected in *Δdrs2* cells, implicating Drs2p as an anchor protein for Gea2p [135]; furthermore, the authors have shown that *Δgea2* cells show morphological defects in the secretory pathway, and are impaired in the initiation of formation of secretory vesicles. Similar interactions have been identified between Neo1p and Ysl2p, a putative GEF for Arl1p (which is an ARF-like GTPase) [129]; *Δysl2* cells are impaired in endocytosis and display a vacuole biogenesis defect [136]; these phenotypes are suppressed by overexpression of Neo1p, implicating a role for Neo1p and Ysl2p in an overlapping pathway. These data suggest a collaborative role for (intracellular localized) type 4 ATPases, ADP-ribosylation factors (ARFs), and guanine nucleotide exchange factors (GEFs) in the assembly of COPI- and/or clathrin-coated vesicles, a process essential for initiation of intracellular-trafficking events.

7.2. The Cdc50p/Lem3p protein family 1

Some of the yeast type 4 ATPases physically interact with members of an evolutionary conserved family of transmembrane proteins, the Cdc50p/Lem3p family 1 [125,137,138] (see Fig. 3). In yeast, this family includes three close homologs, i.e., Lem3p/Ros3p, Cdc50p, and Ynr048wp (recently renamed to Crf1p), which are glycosylated proteins of approximately 60 kDa with two putative transmembrane domains. Lem3p/Ros3p was initially identified in a screen using the Ro09-0198 peptide, an antibiotic which specifically binds to PE [138]. Yeast mutants sensitive to this peptide were mutated in a protein termed Ros3p (Ro-sensitive 3, renamed to Lem3p); green fluorescent protein (GFP)-tagged Lem3p localized to the plasma membrane and the endoplasmic reticulum (ER) [138]. In addition, *ΔLem3* cells were impaired in the ATP-dependent, non-endocytic, uptake of NBD-PE and NBD-PC (but not of NBD-PS) and of alkylphosphocholine drugs [137,138]. Recently, Saito et al. [125] have demonstrated a physical interaction between Lem3p and Dnf1p, and have shown that Lem3p is required for exit of Dnf1p from the ER. Thus, impairment of NBD-PE and NBD-PC translocation in *ΔLem3* cells may be caused by absence of Dnf1p from the plasma membrane. In the same study, Cdc50p has been shown to interact with Drs2p. Both *Δcdc50* and *Δdrs2*

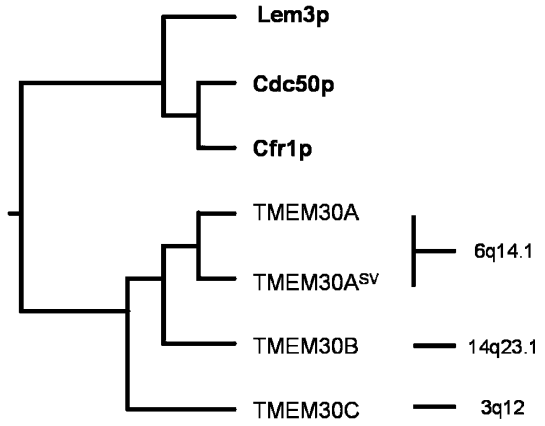

Fig. 3. Phylogenetic analysis of members of the human and yeast *Saccharomyces cerevisiae* Cdc50p/Lem3p family 1. The figure shows the relationship between the full-length proteins of yeast (3 proteins, in bold) and human (3 proteins, including a putative splice variant). The dendrogram was generated using ClustalW analyses. Chromosomal locations of the human family members are indicated. Database accession numbers: Lem3p (NP_014076); Cdc50p (NP_010018); Crf1p/YNR048wp (NP_014446); TMEM30A (transmembrane protein 30A, also termed CDC50A) (NP_060717); TMEM30A^{SV}, possible alternative splice variant of TMEM30A (AAH09006); TMEM30B (transmembrane protein 30B, also termed CDC50B) (XP090844), TMEM30C (transmembrane protein 30C, also termed CDC50C) (no Genbank accession, see [139]).

mutant cells displayed similar phenotypes, including impairment of (polarized) growth, implicating that these proteins interact during the establishment of cell polarity. In analogy with Dnf1p and Lem3p, Drs2p and Cdc50p also localized to the ER in *Δcdc50* and *Δdrs2*, respectively. Furthermore, in endocytosis-mutant cells (*Δvrp1*) both Drs2p and Cdc50p localized to the plasma membrane. Thus, although Drs2p predominantly localizes to the TGN, these data suggest that Drs2p–Cdc50p interaction is required for release of both proteins from the ER and targeting to the TGN, and that the Drs2p–Cdc50p complex cycles between the TGN and the plasma membrane [125]. Using *ΔLem3* and *ΔLem3Δvrp1* cells, the authors demonstrated an APLT activity for the Drs2p–Cdc50p complex: *ΔLem3* cells, lacking plasma membrane expression of Dnf1p, were impaired in NBD-PE and -PC translocation; *ΔLem3Δvrp1* cells, lacking Dnf1p but expressing Drs2p–Cdc50p complex in the plasma membrane, displayed almost normal NBD-PE (but affected NBD-PC) uptake; interestingly, NBD-PS uptake was dramatically increased in *ΔLem3Δvrp1* cells, suggesting an involvement of Drs2p–Cdc50p complex in the translocation of aminophospholipids [125]. Although these studies have indicated that proteins of the Ccd50p/Lem3p family 1 are important accessory factors necessary for proper targeting and activity of type 4 ATPases, the true biochemical activity and cell biological function of these proteins remains to be determined.

Thus far, three highly conserved, human Cdc50p/Lem3p family 1 homologs have been identified in the databases and literature, TMEM30A, TMEM30B, and TMEM30C (TransMEMbrane protein 30A–C, also termed CDC50A–C)

[139], and one putative splice-variant, TMEM30A^{SV} (see Fig. 3). Like their yeast homologs these proteins are glycosylated ~60 kDa proteins with two putative transmembrane domains and a couple of conserved amino acid stretches. These proteins may physically and functionally interact with human type 4 ATPases, although this remains to be substantiated in future research.

8. Concluding remarks

The studies in yeast have greatly contributed to our understanding on the biochemical activities and cell biological roles of several type 4 ATPases. Apparently, type 4 ATPases are important in the establishment of cellular polarity. Functional cellular polarity is essential for proper cell function and is accomplished by intracellular-trafficking events, including exo-, endo-, and transcytosis. Yeast type 4 ATPases are involved in the generation of transport vesicles, and thus are essential in intracellular vesicular and protein trafficking. How these proteins participate in the formation of transport vesicles is still not clear. One explanation may be a role in the translocation of glycerophospholipids in general or aminophospholipids in particular, introducing bilayer imbalance and subsequent membrane curvature which is necessary for vesicle budding and fusion [29,50,52]. Alternatively, these proteins may generate a lipid environment suitable for the anchoring of proteins involved in the initiation of coated-vesicle formation, e.g., ARF GTPases [132]. Evidently, both mechanisms may operate through the same translocation activity.

It is also evident that yeast type 4 ATPases do not act by themselves but rather in a complex. Accessory proteins involved in regulation of trafficking and activity have been described for the Na^+/K^+-ATPase; this protein consists of three subunits, a catalytic α-subunit, a 40- to 60-kDa β-subunit involved in maturation and membrane trafficking, and a small γ-subunit (member of the FXYD protein family) which modulates the activity of the protein [8,140,141]. Indeed, members of the Cdc50p/Lem3p family 1 may be potential β-subunits involved in proper targeting of type 4 ATPases. In addition, other yet to be identified proteins may be involved in the regulation of subcellular expression and activity of type 4 ATPases, e.g., members of the FXYD protein family (reviewed in [140]).

Members of the type 4 subfamily of P-type ATPases are essential in regulating cellular homeostasis, which is underscored by the severity of phenotypes associated with inherited diseases like Byler disease. If one extrapolates the biochemical activities and pathways in which yeast type 4 ATPases participate, type 4-associated human diseases may be caused by defects in bilayer lipid asymmetry, which affects the activities of membrane-associated proteins. As for Byler disease, this may implicate that, due to absence of ATP8B1 and subsequent impaired canalicular membrane asymmetry, the activity of the major bile salt transporter is affected. Alternatively, there may be defects in membrane- and protein-trafficking pathways. Especially in epithelial cells, including hepatocytes and enterocytes, these pathways are essential, as the liver and the intestine are involved in major transcytotic events facilitating uptake, excretion, and/or recycling of numerous substrates. Disturbances in this functional cellular polarity indeed may be the cause of severe and complex human inherited diseases. However, how type 4 ATPases relate to this remains to be elucidated. Study of these proteins is a challenge and will provide further fundamental cell biological insight with regard to membrane dynamics and protein trafficking. In addition, it will help to elucidate the molecular mechanism which underlie severe inherited human diseases such as Byler disease.

References

[1] K.B. Axelsen, M.G. Palmgren, Evolution of substrate specificities in the P-type ATPase superfamily, J. Mol. Evol. 46 (1998) 84–101.
[2] K.B. Axelsen, M.G. Palmgren, Inventory of the superfamily of P-type ion pumps in Arabidopsis, Plant Physiol. 126 (2001) 696–706.
[3] M.G. Palmgren, K.B. Axelsen, Evolution of P-type ATPases, Biochim. Biophys. Acta 1365 (1998) 37–45.
[4] P. Catty, d.A. de Kerchove, A. Goffeau, The complete inventory of the yeast *Saccharomyces cerevisiae* P-type transport ATPases, FEBS Lett. 409 (1997) 325–332.
[5] P.L. Pedersen, E. Carafoli, Ion motive ATPases I. Ubiquity, properties, and significance to cell function, Trends Biochem. Sci. 12 (1987) 146–150.
[6] J.V. Moller, B. Juul, M. le Maire, Structural organization, ion transport, and energy transduction of P-type ATPases., Biochim. Biophys. Acta 1286 (1996) 1–51.
[7] J.C. Skou, The influence of some cations on an adenosine triphosphatase from peripheral nerves, Biochim. Biophys. Acta 23 (1957) 394–401.
[8] J.C. Skou, M. Esmann, The Na,K-ATPase, J. Bioenerg. Biomembr. 24 (1992) 249–261.
[9] M.E. Auland, B.D. Roufogalis, P.F. Devaux, A. Zachowski, Reconstitution of ATP-dependent aminophospholipid translocation in proteoliposomes, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 10938–10942.
[10] T. Pomorski, R. Lombardi, H. Riezman, P.F. Devaux, G. van Meer, J.C. Holthuis, Drs2p-related P-type ATPases Dnf1p and Dnf2p are required for phospholipid translocation across the yeast plasma membrane and serve a role in endocytosis, Mol. Biol. Cell 14 (2003) 1240–1254.
[11] X. Tang, M.S. Halleck, R.A. Schlegel, P. Williamson, A subfamily of P-type ATPases with aminophospholipid transporting activity, Science 272 (1996) 1495–1497.
[12] A. Zachowski, J.P. Henry, P.F. Devaux, Control of transmembrane lipid asymmetry in chromaffin granules by an ATP-dependent protein, Nature 340 (1989) 75–76.
[13] S.R. Cronin, R. Rao, R.Y. Hampton, Cod1p/Spf1p is a P-type ATPase involved in ER function and Ca2+ homeostasis, J. Cell Biol. 157 (2002) 1017–1028.
[14] P.J. Schultheis, T.T. Hagen, K.K. O'Toole, A. Tachibana, C.R. Burke, D.L. McGill, G.W. Okunade, G.E. Shull, Characterization of the P5 subfamily of P-type transport ATPases in mice, Biochem. Biophys. Res. Commun. 323 (2004) 731–738.
[15] S. Vashist, C.G. Frank, C.A. Jakob, D.T. Ng, Two distinctly localized p-type ATPases collaborate to maintain organelle homeostasis

required for glycoprotein processing and quality control, Mol. Biol. Cell 13 (2002) 3955–3966.
[16] O.J ardetzky, Simple allosteric model for membrane pumps, Nature 211 (1966) 969–970.
[17] G.A. Scarborough, Why we must move on from the E1E2 model for the reaction cycle of the P-type ATPases, J. Bioenerg. Biomembr. 35 (2003) 193–201.
[18] G.A. Scarborough, Rethinking the P-type ATPase problem, Trends Biochem. Sci. 28 (2003) 581–584.
[19] D.L. Daleke, J.V. Lyles, Identification and purification of aminophospholipid flippases, Biochim. Biophys. Acta 1486 (2000) 108–127.
[20] T.R. Graham, Flippases and vesicle-mediated protein transport, Trends Cell Biol. 14 (2004) 670–677.
[21] M.S. Halleck, J.F. Lawler Jr., S. Blackshaw, L. Gao, P. Nagarajan, C. Hacker, S. Pyle, J.T. Newman, Y. Nakanishi, H. Ando, D. Weinstock, P. Williamson, R.A. Schlegel, Differential expression of putative transbilayer amphipath transporters, Physiol. Genomics 1 (1999) 139–150.
[22] P. Williamson, R.A. Schlegel, Transbilayer phospholipid movement and the clearance of apoptotic cells, Biochim. Biophys. Acta 1585 (2002) 53–63.
[23] D. Dolis, C. Moreau, A. Zachowski, P.F. Devaux, Aminophospholipid translocase and proteins involved in transmembrane phospholipid traffic, Biophys. Chem. 68 (1997) 221–231.
[24] D. Hoekstra, S.C. Van IJzendoorn, In search of lipid translocases and their biological functions, Dev. Cell 4 (2003) 8–9.
[25] T. Pomorski, J.C. Holthuis, A. Herrmann, G. van Meer, Tracking down lipid flippases and their biological functions, J. Cell Sci. 117 (2004) 805–813.
[26] M.S. Bretscher, Asymmetrical lipid bilayer structure for biological membranes, Nat., New Biol. 236 (1972) 11–12.
[27] S.E. Gordesky, G.V. Marinetti, The asymmetric arrangement of phospholipids in the human erythrocyte membrane, Biochem. Biophys. Res. Commun. 50 (1973) 1027–1031.
[28] A.J. Verkleij, R.F. Zwaal, B. Roelofsen, P. Comfurius, D. Kastelijn, L.L. van Deenen, The asymmetric distribution of phospholipids in the human red cell membrane. A combined study using phospholipases and freeze-etch electron microscopy, Biochim. Biophys. Acta 323 (1973) 178–193.
[29] H. Sprong, S.P. van der, G. van Meer, How proteins move lipids and lipids move proteins, Nat. Rev., Mol. Cell Biol. 2 (2001) 504–513.
[30] D.L. Daleke, Regulation of transbilayer plasma membrane phospholipid asymmetry, J. Lipid Res. 44 (2003) 233–242.
[31] P.F. Devaux, Protein involvement in transmembrane lipid asymmetry, Annu. Rev. Biophys. Biomol. Struct. 21 (1992) 417–439.
[32] A.J. Verkleij, J.A. Post, Membrane phospholipid asymmetry and signal transduction, J. Membr. Biol. 178 (2000) 1–10.
[33] P.F. Devaux, A. Zachowski, G. Morrot, S. Cribier, P. Fellmann, D. Geldwerth, M. Bitbol, P. Herve, Control of the transmembrane phospholipid distribution in eukaryotic cells by aminophospholipid translocase, Biotechnol. Appl. Biochem. 12 (1990) 517–522.
[34] P. Williamson, R.A. Schlegel, Back and forth: the regulation and function of transbilayer phospholipid movement in eukaryotic cells, Mol. Membr. Biol. 11 (1994) 199–216.
[35] E.M. Bevers, P. Comfurius, D.W. Dekkers, R.F. Zwaal, Lipid translocation across the plasma membrane of mammalian cells, Biochim. Biophys. Acta 1439 (1999) 317–330.
[36] W.R. Bishop, R.M. Bell, Assembly of the endoplasmic reticulum phospholipid bilayer: the phosphatidylcholine transporter, Cell 42 (1985) 51–60.
[37] X. Buton, P. Herve, J. Kubelt, A. Tannert, K.N. Burger, P. Fellmann, P. Muller, A. Herrmann, M. Seigneuret, P.F. Devaux, Transbilayer movement of monohexosylsphingolipids in endoplasmic reticulum and Golgi membranes, Biochemistry 41 (2002) 13106–13115.
[38] A. Herrmann, A. Zachowski, P.F. Devaux, Protein-mediated phospholipid translocation in the endoplasmic reticulum with a low lipid specificity, Biochemistry 29 (1990) 2023–2027.
[39] A.K. Menon, W.E. Watkins, S. Hrafnsdottir, Specific proteins are required to translocate phosphatidylcholine bidirectionally across the endoplasmic reticulum, Curr. Biol. 10 (2000) 241–252.
[40] P. Williamson, A. Christie, T. Kohlin, R.A. Schlegel, P. Comfurius, M. Harmsma, R.F. Zwaal, E.M. Bevers, Phospholipid scramblase activation pathways in lymphocytes, Biochemistry 40 (2001) 8065–8072.
[41] R.J. Raggers, T. Pomorski, J.C. Holthuis, N. Kalin, G. van Meer, Lipid traffic: the ABC of transbilayer movement, Traffic 1 (2000) 226–234.
[42] J.J. Smit, A.H. Schinkel, R.P. Oude Elferink, A.K. Groen, E. Wagenaar, L. van Deemter, C.A. Mol, R. Ottenhoff, N.M. van der Lugt, M.A. van Roon, Homozygous disruption of the murine mdr2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease, Cell 75 (1993) 451–462.
[43] A.J. Smith, J.L. Timmermans-Hereijgers, B. Roelofsen, K.W. Wirtz, W.J. van Blitterswijk, J.J. Smit, A.H. Schinkel, P. Borst, The human MDR3 P-glycoprotein promotes translocation of phosphatidylcholine through the plasma membrane of fibroblasts from transgenic mice, FEBS Lett. 354 (1994) 263–266.
[44] A. van Helvoort, A.J. Smith, H. Sprong, I. Fritzsche, A.H. Schinkel, P. Borst, G. van Meer, MDR1 P-glycoprotein is a lipid translocase of broad specificity, while MDR3 P-glycoprotein specifically translocates phosphatidylcholine, Cell 87 (1996) 507–517.
[45] R.J. Raggers, I. Vogels, G. van Meer, Multidrug-resistance P-glycoprotein (MDR1) secretes platelet-activating factor, Biochem. J. 357 (2001) 859–865.
[46] D.W. Dekkers, P. Comfurius, R.G. van Gool, E.M. Bevers, R.F. Zwaal, Multidrug resistance protein 1 regulates lipid asymmetry in erythrocyte membranes, Biochem. J. 350 (Pt. 2) (2000) 531–535.
[47] R.J. Raggers, A. van Helvoort, R. Evers, G. van Meer, The human multidrug resistance protein MRP1 translocates sphingolipid analogs across the plasma membrane, J. Cell Sci. 112 (Pt. 3) (1999) 415–422.
[48] K. Balasubramanian, A.J. Schroit, Aminophospholipid asymmetry: a matter of life and death, Annu. Rev. Physiol. 65 (2003) 701–734.
[49] V.A. Fadok, D. Xue, P. Henson, If phosphatidylserine is the death knell, a new phosphatidylserine-specific receptor is the bellringer, Cell Death Differ. 8 (2001) 582–587.
[50] E. Farge, D.M. Ojcius, A. Subtil, A. Dautry-Varsat, Enhancement of endocytosis due to aminophospholipid transport across the plasma membrane of living cells, Am. J. Physiol. 276 (1999) C725–C733.
[51] R.F. Zwaal, P. Comfurius, E.M. Bevers, Lipid–protein interactions in blood coagulation, Biochim. Biophys. Acta 1376 (1998) 433–453.
[52] P.F. Devaux, Is lipid translocation involved during endo- and exocytosis? Biochimie 82 (2000) 497–509.
[53] M. Seigneuret, P.F. Devaux, ATP-dependent asymmetric distribution of spin-labeled phospholipids in the erythrocyte membrane: relation to shape changes, Proc. Natl. Acad. Sci. U. S. A. 81 (1984) 3751–3755.
[54] U. Marx, T. Polakowski, T. Pomorski, C. Lang, H. Nelson, N. Nelson, A. Herrmann, Rapid transbilayer movement of fluorescent phospholipid analogues in the plasma membrane of endocytosis-deficient yeast cells does not require the Drs2 protein, Eur. J. Biochem. 263 (1999) 254–263.
[55] P. Natarajan, J. Wang, Z. Hua, T.R. Graham, Drs2p-coupled aminophospholipid translocase activity in yeast Golgi membranes and relationship to in vivo function, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 10614–10619.
[56] A. Siegmund, A. Grant, C. Angeletti, L. Malone, J.W. Nichols, H.K. Rudolph, Loss of Drs2p does not abolish transfer of fluorescence-labeled phospholipids across the plasma membrane of *Saccharomyces cerevisiae*, J. Biol. Chem. 273 (1998) 34399–34405.
[57] M.S. Halleck, D. Pradhan, C. Blackman, C. Berkes, P. Williamson, R.A. Schlegel, Multiple members of a third subfamily of P-type

ATPases identified by genomic sequences and ESTs, Genome Res. 8 (1998) 354–361.
[58] I. Mouro, M.S. Halleck, R.A. Schlegel, M.G. Mattei, P. Williamson, A. Zachowski, P. Devaux, J.P. Cartron, Y. Colin, Cloning, expression, and chromosomal mapping of a human ATPase II gene, member of the third subfamily of P-type ATPases and orthologous to the presumed bovine and murine aminophospholipid translocase, Biochem. Biophys. Res. Commun. 257 (1999) 333–339.
[59] M.J. Harris, I.M. Arias, FIC1, a P-type ATPase linked to cholestatic liver disease, has homologues (ATP8B2 and ATP8B3) expressed throughout the body, Biochim. Biophys. Acta 1633 (2003) 127–131.
[60] J. Ding, Z. Wu, B.P. Crider, Y. Ma, X. Li, C. Slaughter, L. Gong, X.S. Xie, Identification and functional expression of four isoforms of ATPase II, the putative aminophospholipid translocase. Effect of isoform variation on the ATPase activity and phospholipid specificity, J. Biol. Chem. 275 (2000) 23378–23386.
[61] L. Wang, C. Beserra, D.L. Garbers, A novel aminophospholipid transporter exclusively expressed in spermatozoa is required for membrane lipid asymmetry and normal fertilization, Dev. Biol. 267 (2004) 203–215.
[62] D.W. Cox, S.D. Moore, Copper transporting P-type ATPases and human disease, J. Bioenerg. Biomembr. 34 (2002) 333–338.
[63] P.C. Bull, G.R. Thomas, J.M. Rommens, J.R. Forbes, D.W. Cox, The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene, Nat. Genet. 5 (1993) 327–337.
[64] J. Chelly, Z. Tumer, T. Tonnesen, A. Petterson, Y. Ishikawa-Brush, N. Tommerup, N. Horn, A.P. Monaco, Isolation of a candidate gene for Menkes disease that encodes a potential heavy metal binding protein, Nat. Genet. 3 (1993) 14–19.
[65] J.F. Mercer, J. Livingston, B. Hall, J.A. Paynter, C. Begy, S. Chandrasekharappa, P. Lockhart, A. Grimes, M. Bhave, D. Siemieniak, Isolation of a partial candidate gene for Menkes disease by positional cloning, Nat. Genet. 3 (1993) 20–25.
[66] K. Petrukhin, S.G. Fischer, M. Pirastu, R.E. Tanzi, I. Chernov, M. Devoto, L.M. Brzustowicz, E. Cayanis, E. Vitale, J.J. Russo, Mapping, cloning and genetic characterization of the region containing the Wilson disease gene, Nat. Genet. 5 (1993) 338–343.
[67] R.E. Tanzi, K. Petrukhin, I. Chernov, J.L. Pellequer, W. Wasco, B. Ross, D.M. Romano, E. Parano, L. Pavone, L.M. Brzustowicz, The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene, Nat. Genet. 5 (1993) 344–350.
[68] C. Vulpe, B. Levinson, S. Whitney, S. Packman, J. Gitschier, Isolation of a candidate gene for Menkes disease and evidence that it encodes a copper-transporting ATPase, Nat. Genet. 3 (1993) 7–13.
[69] A. Odermatt, P.E. Taschner, V.K. Khanna, H.F. Busch, G. Karpati, C.K. Jablecki, M.H. Breuning, D.H. MacLennan, Mutations in the gene-encoding SERCA1, the fast-twitch skeletal muscle sarcoplasmic reticulum Ca2+ ATPase, are associated with Brody disease, Nat. Genet. 14 (1996) 191–194.
[70] A. Sakuntabhai, V. Ruiz-Perez, S. Carter, N. Jacobsen, S. Burge, S. Monk, M. Smith, C.S. Munro, M. O'Donovan, N. Craddock, R. Kucherlapati, J.L. Rees, M. Owen, G.M. Lathrop, A.P. Monaco, T. Strachan, A. Hovnanian, Mutations in ATP2A2, encoding a Ca2+ pump, cause Darier disease, Nat. Genet. 21 (1999) 271–277.
[71] M. Peacocke, A.M. Christiano, Bumps and pumps, SERCA 1999, Nat. Genet. 21 (1999) 252–253.
[72] Z. Hu, J.M. Bonifas, J. Beech, G. Bench, T. Shigihara, H. Ogawa, S. Ikeda, T. Mauro, E.H. Epstein Jr., Mutations in ATP2C1, encoding a calcium pump, cause Hailey–Hailey disease, Nat. Genet. 24 (2000) 61–65.
[73] I.C. Meij, J.B. Koenderink, H. van Bokhoven, K.F. Assink, W.T. Groenestege, J.J. De Pont, R.J. Bindels, L.A. Monnens, L.P. Van Den Heuvel, N.V. Knoers, Dominant isolated renal magnesium loss is caused by misrouting of the Na(+),K(+)-ATPase gamma-subunit, Nat. Genet. 26 (2000) 265–266.
[74] X.L. Sun, D. Li, J. Fang, I. Noyes, B. Casto, K. Theil, C. Shuler, G.E. Milo, Changes in levels of normal ML-1 gene transcripts associated with the conversion of human nontumorigenic to tumorigenic phenotypes, Gene Expr. 8 (1999) 129–139.
[75] B.D. Bower, P.M. Jeavons, The "happy puppet" syndrome, Arch. Dis. Child. 42 (1967) 298–302.
[76] C.A. Williams, H. Angelman, J. Clayton-Smith, D.J. Driscoll, J.E. Hendrickson, J.H. Knoll, R.E. Magenis, A. Schinzel, J. Wagstaff, E.M. Whidden, Angelman syndrome: consensus for diagnostic criteria. Angelman Syndrome Foundation, Am. J. Med. Genet. 56 (1995) 237–238.
[77] T. Kishino, M. Lalande, J. Wagstaff, UBE3A/E6-AP mutations cause Angelman syndrome, Nat. Genet. 15 (1997) 70–73.
[78] M. Meguro, A. Kashiwagi, K. Mitsuya, M. Nakao, I. Kondo, S. Saitoh, M. Oshimura, A novel maternally expressed gene, ATP10C, encodes a putative aminophospholipid translocase associated with Angelman syndrome, Nat. Genet. 28 (2001) 19–20.
[79] Y.H. Jiang, D. Armstrong, U. Albrecht, C.M. Atkins, J.L. Noebels, G. Eichele, J.D. Sweatt, A.L. Beaudet, Mutation of the Angelman ubiquitin ligase in mice causes increased cytoplasmic p53 and deficits of contextual learning and long-term potentiation, Neuron 21 (1998) 799–811.
[80] M. Dhar, L.S. Webb, L. Smith, L. Hauser, D. Johnson, D.B. West, A novel ATPase on mouse chromosome 7 is a candidate gene for increased body fat, Physiol. Genomics 4 (2000) 93–100.
[81] M. Dhar, L. Hauser, D. Johnson, An aminophospholipid translocase associated with body fat and type 2 diabetes phenotypes, Obes. Res. 10 (2002) 695–702.
[82] M.S. Dhar, C.S. Sommardahl, T. Kirkland, S. Nelson, R. Donnell, D.K. Johnson, L.W. Castellani, Mice heterozygous for Atp10c, a putative amphipath, represent a novel model of obesity and type 2 diabetes, J. Nutr. 134 (2004) 799–805.
[83] S. Flamant, P. Pescher, B. Lemercier, M. Clement-Ziza, F. Kepes, M. Fellous, G. Milon, G. Marchal, C. Besmond, Characterization of a putative type IV aminophospholipid transporter P-type ATPase, Mamm. Genome 14 (2003) 21–30.
[84] S. Collins, T.L. Martin, R.S. Surwit, J. Robidoux, Genetic vulnerability to diet-induced obesity in the C57BL/6J mouse: physiological and molecular characteristics, Physiol. Behav. 81 (2004) 243–248.
[85] R.S. Surwit, M.N. Feinglos, J. Rodin, A. Sutherland, A.E. Petro, E.C. Opara, C.M. Kuhn, M. Rebuffe-Scrive, Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice, Metabolism 44 (1995) 645–651.
[86] N.M. Andrew, M.R. Bowl, B. Harding, D. Schlessinger, M.P. Whyte, R.V. Thakker, X-linked hypoparathyroidism region on Xq27 is evolutionarily conserved with regions on 3q26 and 13q34 and contains a novel P-type ATPase, Genomics 84 (2004) 1060–1070.
[87] L.N. Bull, M.J. van Eijk, L. Pawlikowska, J.A. DeYoung, J.A. Juijn, M. Liao, L.W. Klomp, N. Lomri, R. Berger, B.F. Scharschmidt, A.S. Knisely, R.H. Houwen, N.B. Freimer, A gene encoding a P-type ATPase mutated in two forms of hereditary cholestasis, Nat. Genet. 18 (1998) 219–224.
[88] R.J. Clayton, F.L. Iber, B.H. Ruebner, V.A. McKusick, Byler disease. Fatal familial intrahepatic cholestasis in an Amish kindred, Am. J. Dis. Child. 117 (1969) 112–124.
[89] P.L. Jansen, M.M. Muller, Progressive familial intrahepatic cholestasis types 1, 2, and 3, Gut 42 (1998) 766–767.
[90] L.W. Klomp, L.N. Bull, A.S. Knisely, M.A. Der Doelen, J.A. Juijn, R. Berger, S. Forget, I.M. Nielsen, H. Eiberg, R.H. Houwen, A missense mutation in FIC1 is associated with greenland familial cholestasis, Hepatology 32 (2000) 1337–1341.
[91] A.S. Knisely, Progressive familial intrahepatic cholestasis: a personal perspective, Pediatr. Dev. Pathol. 3 (2000) 113–125.
[92] S.W. van Mil, L.W. Klomp, L.N. Bull, R.H. Houwen, FIC1 disease: a spectrum of intrahepatic cholestatic disorders, Semin. Liver Dis. 21 (2001) 535–544.
[93] W.H. Summerskill, J.M. Walshe, Benign recurrent intrahepatic "obstructive" jaundice, Lancet 2 (1959) 686–690.
[94] L.W. Klomp, J.C. Vargas, S.W. van Mil, L. Pawlikowska, S.S.

Strautnieks, M.J. van Eijk, J.A. Juijn, C. Pabon-Pena, L.B. Smith, J.A. DeYoung, J.A. Byrne, J. Gombert, B.G. van der, R. Berger, I. Jankowska, J. Pawlowska, E. Villa, A.S. Knisely, R.J. Thompson, N.B. Freimer, R.H. Houwen, L.N. Bull, Characterization of mutations in ATP8B1 associated with hereditary cholestasis, Hepatology 40 (2004) 27–38.

[95] E.M. Alonso, D.C. Snover, A. Montag, D.K. Freese, P.F. Whitington, Histologic pathology of the liver in progressive familial intrahepatic cholestasis, J. Pediatr. Gastroenterol. Nutr. 18 (1994) 128–133.

[96] L.N. Bull, V.E. Carlton, N.L. Stricker, S. Baharloo, J.A. DeYoung, N.B. Freimer, M.S. Magid, E. Kahn, J. Markowitz, F.J. DiCarlo, L. McLoughlin, J.T. Boyle, B.B. Dahms, P.R. Faught, J.F. Fitzgerald, D.A. Piccoli, C.L. Witzleben, N.C. O'Connell, K.D. Setchell, R.M. Agostini Jr., S.A. Kocoshis, J. Reyes, A.S. Knisely, Genetic and morphological findings in progressive familial intrahepatic cholestasis (Byler disease [PFIC-1] and Byler syndrome): evidence for heterogeneity, Hepatology 26 (1997) 155–164.

[97] F.X. Felberbauer, G. Amann, W. Rebhandl, W.D. Huber, Follow-up after partial external biliary diversion in familial cholestasis of infancy, J. Pediatr. Gastroenterol. Nutr. 31 (2000) 322.

[98] A.C. Kurbegov, K.D. Setchell, J.E. Haas, G.W. Mierau, M. Narkewicz, J.D. Bancroft, F. Karrer, R.J. Sokol, Biliary diversion for progressive familial intrahepatic cholestasis: improved liver morphology and bile acid profile, Gastroenterology 125 (2003) 1227–1234.

[99] T. Oshima, K. Ikeda, T. Takasaka, Sensorineural hearing loss associated with Byler disease, Tohoku J. Exp. Med. 187 (1999) 83–88.

[100] P. Lykavieris, S. van Mil, D. Cresteil, M. Fabre, M. Hadchouel, L. Klomp, O. Bernard, E. Jacquemin, Progressive familial intrahepatic cholestasis type 1 and extrahepatic features: no catch-up of stature growth, exacerbation of diarrhea, and appearance of liver steatosis after liver transplantation, J. Hepatol. 39 (2003) 447–452.

[101] E.F. Eppens, S.W. van Mil, J.M. de Vree, K.S. Mok, J.A. Juijn, R.P. Oude Elferink, R. Berger, R.H. Houwen, L.W. Klomp, FIC1, the protein affected in two forms of hereditary cholestasis, is localized in the cholangiocyte and the canalicular membrane of the hepatocyte, J. Hepatol. 35 (2001) 436–443.

[102] P. Ujhazy, D. Ortiz, S. Misra, S. Li, J. Moseley, H. Jones, I.M. Arias, Familial intrahepatic cholestasis 1: studies of localization and function, Hepatology 34 (2001) 768–775.

[103] S.W. van Mil, M.M. van Oort, I. d.B. van, R. Berger, R.H. Houwen, L.W. Klomp, Fic1 is expressed at apical membranes of different epithelial cells in the digestive tract and is induced in the small intestine during postnatal development of mice, Pediatr. Res. 56 (2004) 981–987.

[104] E. Jacquemin, M. Dumont, O. Bernard, S. Erlinger, M. Hadchouel, Evidence for defective primary bile acid secretion in children with progressive familial intrahepatic cholestasis (Byler disease), Eur. J. Pediatr. 153 (1994) 424–428.

[105] Y. Tazawa, M. Yamada, M. Nakagawa, T. Konno, K. Tada, Bile acid profiles in siblings with progressive intrahepatic cholestasis: absence of biliary chenodeoxycholate, J. Pediatr. Gastroenterol. Nutr. 4 (1985) 32–37.

[106] J.C. Emond, P.F. Whitington, Selective surgical management of progressive familial intrahepatic cholestasis (Byler's disease), J. Pediatr. Surg. 30 (1995) 1635–1641.

[107] V.L. Ng, F.C. Ryckman, G. Porta, I.K. Miura, E. de Carvalho, M.F. Servidoni, J.A. Bezerra, W.F. Balistreri, Long-term outcome after partial external biliary diversion for intractable pruritus in patients with intrahepatic cholestasis, J. Pediatr. Gastroenterol. Nutr. 30 (2000) 152–156.

[108] P.F. Whitington, G.L. Whitington, Partial external diversion of bile for the treatment of intractable pruritus associated with intrahepatic cholestasis, Gastroenterology 95 (1988) 130–136.

[109] M. Makishima, A.Y. Okamoto, J.J. Repa, H. Tu, R.M. Learned, A. Luk, M.V. Hull, K.D. Lustig, D.J. Mangelsdorf, B. Shan, Identification of a nuclear receptor for bile acids, Science 284 (1999) 1362–1365.

[110] D.J. Parks, S.G. Blanchard, R.K. Bledsoe, G. Chandra, T.G. Consler, S.A. Kliewer, J.B. Stimmel, T.M. Willson, A.M. Zavacki, D.D. Moore, J.M. Lehmann, Bile acids: natural ligands for an orphan nuclear receptor, Science 284 (1999) 1365–1368.

[111] F. Kuipers, T. Claudel, E. Sturm, B. Staels, The Farnesoid X Receptor (FXR) as modulator of bile acid metabolism, Rev. Endocr. Metab. Disord. 5 (2004) 319–326.

[112] J.J. Eloranta, G.A. Kullak-Ublick, Coordinate transcriptional regulation of bile acid homeostasis and drug metabolism, Arch. Biochem. Biophys. 433 (2005) 397–412.

[113] G.A. Kullak-Ublick, B. Stieger, P.J. Meier, Enterohepatic bile salt transporters in normal physiology and liver disease, Gastroenterology 126 (2004) 322–342.

[114] H. Li, F. Chen, Q. Shang, L. Pan, B.L. Shneider, J.Y. Chiang, B.M. Forman, M. Ananthanarayanan, G.S. Tint, G. Salen, G. Xu, FXR-activating ligands inhibit rabbit ASBT expression via FXR-SHP-FTF cascade, Am. J. Physiol.: Gastrointest. Liver. Physiol. 288 (2005) G60–G66.

[115] E. Neimark, F. Chen, X. Li, B.L. Shneider, Bile acid-induced negative feedback regulation of the human ileal bile acid transporter, Hepatology 40 (2004) 149–156.

[116] L. Alvarez, P. Jara, E. Sanchez-Sabate, L. Hierro, J. Larrauri, M.C. Diaz, C. Camarena, l. De, V.E. Frauca, E. Lopez-Collazo, P. Lapunzina, Reduced hepatic expression of farnesoid X receptor in hereditary cholestasis associated to mutation in ATP8B1, Hum. Mol. Genet. 13 (2004) 2451–2460.

[117] F. Chen, M. Ananthanarayanan, S. Emre, E. Neimark, L.N. Bull, A.S. Knisely, S.S. Strautnieks, R.J. Thompson, M.S. Magid, R. Gordon, N. Balasubramanian, F.J. Suchy, B.L. Shneider, Progressive familial intrahepatic cholestasis, type 1, is associated with decreased farnesoid X receptor activity, Gastroenterology 126 (2004) 756–764.

[118] C. Demeilliers, E. Jacquemin, V. Barbu, M. Mergey, L. Fouassier, Abnormal hepatobiliary expression of genes involved in bile secretion in progressive familial intrahepatic cholestasis of type 1, Hepatology 40 (4 Suppl. 1) (2004) 467A.

[119] L. Pawlikowska, A. Groen, E.F. Eppens, C. Kunne, R. Ottenhoff, N. Looije, A.S. Knisely, N.P. Killeen, L.N. Bull, R.P. Elferink, N.B. Freimer, A mouse genetic model for familial cholestasis caused by ATP8B1 mutations reveals perturbed bile salt homeostasis but no impairment in bile secretion, Hum. Mol. Genet. 13 (2004) 881–892.

[120] A. Tannert, D. Wustner, J. Bechstein, P. Muller, P.F. Devaux, A. Herrmann, Aminophospholipids have no access to the luminal side of the biliary canaliculus: implications for the specific lipid composition of the bile fluid, J. Biol. Chem. 278 (2003) 40631–40639.

[121] K. Hanada, R.E. Pagano, A Chinese hamster ovary cell mutant defective in the non-endocytic uptake of fluorescent analogs of phosphatidylserine: isolation using a cytosol acidification protocol, J. Cell Biol. 128 (1995) 793–804.

[122] Z. Hua, P. Fatheddin, T.R. Graham, An essential subfamily of Drs2p-related P-type ATPases is required for protein trafficking between Golgi complex and endosomal/vacuolar system, Mol. Biol. Cell 13 (2002) 3162–3177.

[123] C.Y. Chen, M.F. Ingram, P.H. Rosal, T.R. Graham, Role for Drs2p, a P-type ATPase and potential aminophospholipid translocase, in yeast late Golgi function, J. Cell Biol. 147 (1999) 1223–1236.

[124] W.E. Gall, N.C. Geething, Z. Hua, M.F. Ingram, K. Liu, S.I. Chen, T.R. Graham, Drs2p-dependent formation of exocytic clathrin-coated vesicles in vivo, Curr. Biol. 12 (2002) 1623–1627.

[125] K. Saito, K. Fujimura-Kamada, N. Furuta, U. Kato, M. Umeda, K. Tanaka, Cdc50p, a protein required for polarized growth, associates with the Drs2p P-type ATPase implicated in phospholipid translocation in *Saccharomyces cerevisiae*, Mol. Biol. Cell 15 (2004) 3418–3432.

[126] P. Novick, C. Field, R. Schekman, Identification of 23 complemen-

tation groups required for post-translational events in the yeast secretory pathway, Cell 21 (1980) 205–215.
[127] F. Reggiori, C.W. Wang, U. Nair, T. Shintani, H. Abeliovich, D.J. Klionsky, Early stages of the secretory pathway, but not endosomes, are required for Cvt vesicle and autophagosome assembly in *Saccharomyces cerevisiae*, Mol. Biol. Cell 15 (2004) 2189–2204.
[128] T.R. Prezant, W.E. Chaltraw Jr., N. Fischel-Ghodsian, Identification of an overexpressed yeast gene which prevents aminoglycoside toxicity, Microbiology 142 (Pt. 12) (1996) 3407–3414.
[129] S. Wicky, H. Schwarz, B. Singer-Kruger, Molecular interactions of yeast Neo1p, an essential member of the Drs2 family of amino-phospholipid translocases, and its role in membrane trafficking within the endomembrane system, Mol. Cell. Biol. 24 (2004) 7402–7418.
[130] Z. Hua, T.R. Graham, Requirement for neo1p in retrograde transport from the Golgi complex to the endoplasmic reticulum, Mol. Biol. Cell 14 (2003) 4971–4983.
[131] J.G. Donaldson, C.L. Jackson, Regulators and effectors of the ARF GTPases, Curr. Opin. Cell Biol. 12 (2000) 475–482.
[132] T. Kirchhausen, Three ways to make a vesicle, Nat. Rev., Mol. Cell Biol. 1 (2000) 187–198.
[133] E.C. Gaynor, C.Y. Chen, S.D. Emr, T.R. Graham, ARF is required for maintenance of yeast Golgi and endosome structure and function, Mol. Biol. Cell 9 (1998) 653–670.
[134] T. Stearns, M.C. Willingham, D. Botstein, R.A. Kahn, ADP-ribosylation factor is functionally and physically associated with the Golgi complex, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 1238–1242.
[135] S. Chantalat, S.K. Park, Z. Hua, K. Liu, R. Gobin, A. Peyroche, A. Rambourg, T.R. Graham, C.L. Jackson, The Arf activator Gea2p and the P-type ATPase Drs2p interact at the Golgi in *Saccharomyces cerevisiae*, J. Cell Sci. 117 (2004) 711–722.
[136] A. Jochum, D. Jackson, H. Schwarz, R. Pipkorn, B. Singer-Kruger, Yeast Ysl2p, homologous to Sec7 domain guanine nucleotide exchange factors, functions in endocytosis and maintenance of vacuole integrity and interacts with the Arf-Like small GTPase Arl1p, Mol. Cell. Biol. 22 (2002) 4914–4928.
[137] P.K. Hanson, L. Malone, J.L. Birchmore, J.W. Nichols, Lem3p is essential for the uptake and potency of alkylphosphocholine drugs, edelfosine and miltefosine, J. Biol. Chem. 278 (2003) 36041–36050.
[138] U. Kato, K. Emoto, C. Fredriksson, H. Nakamura, A. Ohta, T. Kobayashi, K. Murakami-Murofushi, T. Kobayashi, M. Umeda, A novel membrane protein, Ros3p, is required for phospholipid translocation across the plasma membrane in *Saccharomyces cerevisiae*, J. Biol. Chem. 277 (2002) 37855–37862.
[139] Y. Katoh, M. Katoh, Identification and characterization of CDC50A, CDC50B and CDC50C genes in silico, Oncol. Rep. 12 (2004) 939–943.
[140] K. Geering, P. Beguin, H. Garty, S. Karlish, M. Fuzesi, J.D. Horisberger, G. Crambert, FXYD proteins: new tissue- and isoform-specific regulators of Na, K-ATPase, Ann. N. Y. Acad. Sci. 986 (2003) 388–394.
[141] A.G. Therien, S.J. Karlish, R. Blostein, Expression and functional role of the gamma subunit of the Na,K-ATPase in mammalian cells, J. Biol. Chem. 274 (1999) 12252–12256.
[142] T. Nagase, R. Kikuno, O. Ohara, Prediction of the coding sequences of unidentified human genes: XXII. The complete sequences of 50 new cDNA clones which code for large proteins, DNA Res. 8 (2001) 319–327.
[143] K. Ishikawa, T. Nagase, M. Suyama, N. Miyajima, A. Tanaka, H. Kotani, N. Nomura, O. Ohara, Prediction of the coding sequences of unidentified human genes: X. The complete sequences of 100 new cDNA clones from brain which can code for large proteins in vitro, DNA Res. 5 (1998) 169–176.
[144] T. Nagase, K. Ishikawa, M. Suyama, R. Kikuno, N. Miyajima, A. Tanaka, H. Kotani, N. Nomura, O. Ohara, Prediction of the coding sequences of unidentified human genes: XI. The complete sequences of 100 new cDNA clones from brain which code for large proteins in vitro, DNA Res. 5 (1998) 277–286.
[145] T. Nagase, R. Kikuno, K. Ishikawa, M. Hirosawa, O. Ohara, Prediction of the coding sequences of unidentified human genes: XVII. The complete sequences of 100 new cDNA clones from brain which code for large proteins in vitro, DNA Res. 7 (2000) 143–150.
[146] R. Kikuno, T. Nagase, K. Ishikawa, M. Hirosawa, N. Miyajima, A. Tanaka, H. Kotani, N. Nomura, O. Ohara, Prediction of the coding sequences of unidentified human genes: XIV. The complete sequences of 100 new cDNA clones from brain which code for large proteins in vitro, DNA Res. 6 (1999) 197–205.
[147] T. Nagase, K. Ishikawa, M. Suyama, R. Kikuno, M. Hirosawa, N. Miyajima, A. Tanaka, H. Kotani, N. Nomura, O. Ohara, Prediction of the coding sequences of unidentified human genes: XIII. The complete sequences of 100 new cDNA clones from brain which code for large proteins in vitro, DNA Res. 6 (1999) 63–70.